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Applicants:	Hubert Dorn et al.
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Hon.Commissioner of Patents and Trademarks
Washington, D.C. 20231

DECLARATION

Dr.Hubert Dorn of Pahlkestrasse 71, 42115 Wuppertal, Germany, hereby declares

- that he is a veterinarian having studied at the Universities of Hannover and Giessen;

- that in 1970 he received his doctor's degree at the University of Hannover;

- that in 1970 he entered clinical development department of business group animal health of Bayer AG;

- that in 1990 he became head of clinical development for pet products and in 1992 he became head of clinical development for livestock and pet products of Bayer AG's animal health business group;

- that he is one of the inventors of the present patent application;

- that under his supervision product development work for flea combatting products for cats and dogs has been conceived, planned and carried out either within Bayer's research and development facilities or with outside research groups;

- that under his supervision clinical development of Bayer's insecticide Imidacloprid as a flea combatting product for cats and dogs has been carried out;

- that part of this work was directed to comparison with products of other companies either already on the market for this use or also under development;

- that the following reports have been produced under his guidance and supervision

    1. A comparative clinical examination of the activity and tolerance of oral formulations of imidacloprid and three additional chemically analogous substances by experimentally infesting dogs with fleas

    2. The spot-on mode of action of 10 % spot-on imidacloprid against the infestation of dogs with fleas (Ctenocephalides felis)

    3. Assessment of the use of imidacloprid to prevent and treat flea infestation caused by Ctenocephalides felis felis in cats

Report 1

A comparative clinical examination of the activity and tolerance of oral formulations of imidacloprid and three additional chemically analogous substances by experimentally infesting dogs with fleas

The aim of the present investigation is to examine the activity and tolerance of oral formulations of four chloronicotinyl guanidines using dogs as the target animal species, a fixed dosage and five test groups.

The tests were carried out on 12 dogs (beagles). Since four substances were tested, each test group consisted of 3 animals. 4 additional animals, which were used as controls, had been pretreated with pyriproxyfen.

The following table summarises the animal data relevant for the performance of the study.

Table 1: Animal data

animal no.	sex	weight (kg)
NYAA 255 4283	m	13.4
NYAA 253 2522	m	12.3
NYAA 254 5136	m	13.6
NYAA 254 7104	f	13.7
NYAA 254 9018	f	11.6
NYAA 254 7899	f	10.5
NYAA 255 5042	m	11.2
NYAA 253 1640	m	13.4
NYAA 255 3376	m	12.1
NYAA 254 4768	m	14.1
NYAA 255 4674	f	10.9
NYAA 255 1039	f	10.5
93 4875	f	13.1
94 5732	m	12.6
93 4876	f	13.2
93 4794	f	15.2

The animals were randomised on the basis of the degree of their infestation with fleas. For this purpose the animals were infested with fleas twice before the treatment. On test day 0 the dogs were classified according to the degree of their infestation with fleas and distributed evenly among the various test groups. The control animals were selected according to the degree of their infestation with fleas.

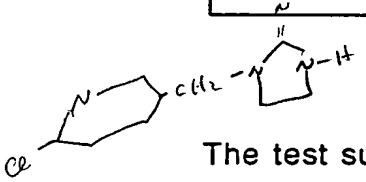
At specific intervals before and after the treatment each dog was infested with approximately 100 fleas [cat fleas, C. felis (Siphonaptera: Pulicidae) - unfed imagoes no more than 4 weeks old] in the inside thigh area. The acceptance by the host was monitored. The corresponding intervals at which the individual preinfestations and the subsequent reinfestations were carried out are shown in the table at the end of this chapter.

The test substances were administered once in the form of tablets at a dosage of 10 mg/kg body weight for all treated groups.

The following table lists the groups into which the animals were divided and the dosages of the corresponding substances administered to each individual animal.

Table 2: Overview of the test groups and the treatment employed

test group	animal no.	weight (kg)	dosage	
			mg	no. of tablets
imidacloprid	NYAA 255 4283	13.4	130	6.5
	NYAA 253 2522	12.3	120	6
	NYAA 254 5136	13.6	140	7
TI 304	NYAA 254 7104	13.7	140	7
	NYAA 254 9018	11.6	120	6
	NYAA 254 7899	10.5	110	5.5
TI 435	NYAA 255 5042	11.2	110	5.5
	NYAA 253 1640	13.4	120	6
	NYAA 255 3376	12.1	120	6
AKD 1022	NYAA 254 4768	14.1	140	7
	NYAA 255 4674	10.9	110	5.5
	NYAA 255 1039	10.5	110	5.5
control	93 4875	13.1	-	-
	94 5732	12.6	-	-
	93 4876	13.2	-	-
	93 4794	15.2	-	-



The test substances to be examined belong to the nicotinyl nitroguanidine class of compounds. The substances were in the form of 120 mg tablets each containing 20 mg of active compound.

I. imidacloprid 1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylidene amine

II. TI 304 N-[(6-chloro-3-pyridinyl)methyl]-N-ethyl-N-methyl-2-nitro-1,2-ethene diamine

III. TI 435 1-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

IV. AKD 1022 1-[(2-chloro-5-thiazolyl)methyl]-tetrahydro-3,5-dimethyl-N-nitro-1,3,5-triazin-2-imine

In order to examine the activity and tolerance of the treatment all the animals of the individual treatment groups and the control groups were subjected to a general clinical examination and a special clinical examination for their infestation with fleas 24, 48 and 72 hours after the treatment. This procedure was repeated after the weekly reinfestation (see Table 3). Tolerance was assessed qualitatively (individual description of the findings determined).

Table 3: The time schedule of the tests

1: test group 1 (imidacloprid) 2: test group 2 (TI 304) 3: test group 3 (TI 435)
4: test group 4 (AKD 1022) 5: test group 5 (control)

day of test	date	determination of weight	randomisation	infestation	treatment	clinical examination
-7	12.3.96			1, 2, 3, 4, 5		
-3	15.3.96			1, 2, 3, 4, 5		
-1	18.3.96	1, 2, 3, 4, 5				
0	19.3.96	1, 2, 3, 4, 5	1, 2, 3, 4, 5		1, 2, 3, 4, 5	1, 2, 3, 4, 5
1	20.3.96					1, 2, 3, 4, 5
2	21.3.96					1, 2, 3, 4, 5
3	22.3.96					1, 2, 3, 4, 5
7	26.3.96			1, 2, 3, 4, 5		
8	27.3.96					1, 2, 3, 4, 5
9	28.3.96					1, 2, 3, 4, 5
10	29.3.96					1, 2, 3, 4, 5

Results and discussion

All of the substances tested displayed a very high immediate activity. On test day 1 (24 hours after the administration of the substances) all the animals in the treatment groups were free from fleas.

The flea infestation rates on test day 8 (24 hours after reinfestation) indicate that only a small degree of residual activity exists which no longer produces any major reduction in the infestation of the treated animals with fleas. Also the activity between day 8 (24 hours after reinfestation) and day 10 (72 hours after reinfestation) no longer reveals any insecticidal activity of the substances tested.

The clinical tolerance of the oral formulations was very high. The clinical examination of the animals did not provide any indication of intolerance reactions.

The duration of activity is therefore between 1-7 days for all of the substances. The assessment of the duration of activity could only be carried out approximately for methodological reasons, since reinfestation was not carried out until 7 days after the treatment. No major differences in activity or tolerance were determined between the individual substances.

All the results obtained relate to the formulations tested with a dosage of 10 mg/kg. It is thus unlikely that adequate protection against infestation with fleas can be guaranteed by a single dose of <10mg/kg of the formulations in question.

Report 2

The spot-on mode of action of 10% spot-on imidacloprid against the infestation of dogs with fleas (Ctenocephalides felis)

On day 0, 3 beagles were each treated dermally between their shoulder blades with 0.1 ml/kg body weight of 10% spot-on imidacloprid (10 mg/kg body weight). 3 additional beagles were not treated and were used as control dogs.

On day 5, 3 flea cages containing cat fleas were attached to specially prepared areas of skin of the thorax region of each of the 6 beagles:

I one flea cage per dog was attached directly to skin which had been cleaned with propylene carbonate, washed with soap and then shorn and shaven;

II one flea cage per dog was attached directly to shorn and shaven skin which had not be cleaned with propylene carbonate or washed;

III one flea cage per dog was attached to fur or skin which had not been cleaned with propylene carbonate or washed.

On day 6, 7 and 8 the fleas were examined for their vitality and sucking activity.

The following observations were made with regard to the fleas on the dogs treated with imidacloprid, 3 days after they had been applied: Those fleas on skin (I) which was substantially free of imidacloprid were only slightly affected after 3 days on the host (their movements were slower than on the control dogs) and a large amount of blood had been sucked (although not as much as in the control dogs). After 3 days almost no blood had however been sucked by the fleas on the skin (II) containing imidacloprid or on the fur

and skin(III) containing imidacloprid and the mortality was almost 100% (the few fleas which were still alive were severely affected).

In all three cases (I; II and III) the fleas on the control dogs had not been affected at all and had sucked a large amount of blood after 3 days.

beagles	flea cage	day 6 (24 h after the attachment of the flea cages)	day 7 (48 h after the attachment of the flea cages)	day 8 (72 h after the attachment of the flea cages)
3 beagles treated with imidacloprid	I	flea excrement + all of the fleas alive	flea excrement ++ all of the fleas alive	flea excrement +++ all of the fleas alive, but slightly affected (they move more slowly than on the control animals)
	II	flea excrement - some of the fleas dead	flea excrement - the majority of the fleas dead	flea excement - (in some cases only very small amounts present) all of the fleas dead (a few fleas are still alive, but they are severely affected)
	III	flea excrement - no fleas visible in the fur	flea excrement - no fleas visible in the fur	flea excrement - (in some cases only very small amounts present) all of the fleas dead (a few fleas are still alive, but they are severely affected)
3 untreated control dogs	I	flea excrement ++ all of the fleas alive	flea excrement +++ all of the fleas alive	flea excrement ++++ all of the fleas alive and unaffected
	II	flea excrement ++ all of the fleas alive	flea excrement +++ all of the fleas alive	flea excrement ++++ all of the fleas alive and unaffected
	III	flea excrement ++ no fleas visible in the fur	flea excrement +++ no fleas visible in the fur	flea excrement ++++ all of the fleas alive and unaffected

I = fleas on shorn, shaven skin which has been cleaned with propylene carbonate and washed with soap

II = fleas on shorn, shaven skin which has not been cleaned with propylene carbonate or washed

III = fleas on hairy skin which has not been cleaned with propylene carbonate or washed

On day 0, 3 beagles were each treated dermally between the shoulder blades with 0.1 ml/kg of 10% spot-on imidacloprid (10 mg/kg). 3 additional beagles were not treated and were used as controls. On day 5, 3 flea cages

containing cat fleas were attached to specially prepared areas of skin of the thorax region of each of the 6 beagles:

I one flea cage per dog was attached directly to skin which had been cleaned with propylene carbonate and washed with soap and then shorn and shaven;

II one flea cage per dog was attached directly to shorn and shaven skin which had not be cleaned with propylene carbonate or washed;

III one flea cage per dog was attached to fur or skin which had not been cleaned with propylene carbonate or washed.

On day 6, 7 and 8 the fleas were examined for their vitality and sucking activity.

The fleas in flea cages I, II and III on the control dogs were not affected at all and displayed a high degree of sucking activity over the period of observation.

The fleas in flea cage I on the dogs treated with imidacloprid were only slightly affected and displayed a high degree of sucking activity over the period of observation.

By contrast, the fleas in flea cages II and III on the dogs treated with imidacloprid displayed a mortality of almost 100 % at the end of the period of observation and practically no excrement had been deposited.

These results reveal that the active compound present on the skin and in the fur of animals plays a crucial role. The mere presence of imidacloprid on the skin seems to be sufficient to ensure a 100 % mortality of the fleas (there is no significant difference between the fleas in flea cages II and III). The

amount of imidacloprid merely consumed orally by the fleas via the blood does not appear to be sufficient to ensure the destruction of the fleas within 3 days. Thus 10% spot-on imidacloprid appears to have very high contact activity on being applied dermally to dogs. This is above all due to the active compound present on the skin, whereas the oral effect is minimal.

The slight effect on the fleas which was nevertheless observed in flea cages I on the dogs treated with imidacloprid - including the smaller amount of blood sucked compared with the controls - could be due to imidacloprid still being present on the skin, despite cleaning. It is however also possible that the quantity of active compound consumed orally by the fleas after sucking blood for three days is sufficient to affect the fleas slightly.

Report 3

ASSESSMENT OF THE USE OF IMIDACLOPRID TO PREVENT AND TREAT FLEA INFESTATION

CAUSED BY Ctenocephalides felis felis

IN CATS

INTRODUCTION

Imidacloprid (1-6(-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine) is a chloronicotinyl nitroguanidine compound that was originally developed as an insecticide for plant use.

This clinical expert report has been written to assess the tolerance and efficacy profile of Imidacloprid, in the proposed final formulation, for the treatment and control of fleas on cats.

THE FLEA PROBLEM

Fleas on domestic pets are becoming recognised as an increasing problem, despite the availability of numerous "flea remedies". There are several possible reasons for this, firstly that there is a growing cat and dog population. For example, the Pet Food Manufacturers Association in the UK reported that the cat population had increased by 33% between 1981 and 1991. During the same time the dog population had increased by 22%. Many of these dogs and cats live in our homes which, especially with central heating, are now maintained at higher ambient temperatures than was previously possible. Additional features such as fitted carpets, upholstered furniture and draught-proofing have been cited as factors that may assist in maintaining flea populations in domestic accommodation.

Finally, recent mild, humid winters have prevented flea numbers from being drastically reduced as occurs in dry, cold weather. Therefore, after such mild winters, there is a substantial baseline population.

The majority of skin conditions seen in small animal, first opinion practice are related to flea infestation. The most commonly observed clinical sign of flea infestation in cats (and dogs) is caused by the development of an allergy to flea saliva, injected as the fleas bit, resulting in flea allergic dermatitis. This is a distressing skin condition which, as far as can be ascertained from the current literature, will flare up again in a sensitised individual whenever that individual is exposed to a threshold number of fleas. It is therefore, crucial to adopt a flea population reduction policy in these situations. Fleas are also responsible for the transmission of diseases, for example the flea acts as an intermediate host for the tapeworm, Dipylidium caninum.

Fleas, particularly unfed cat (Ctenocephalides felis, felis), are zoonoses as they will bite humans. Some humans develop a hypersensitivity reaction to these bites in a similar way to dogs or cats. Fleas may also transmit disease to humans, for example the literature contains reports of human infection with Dipylidium caninum. It may therefore be appreciated that, apart from any aesthetic concerns for avoiding having flea-infested pets, fleas can pose a threat to animal and human health.

The most commonly found flea on cats is the cat flea, Ctenocephalides felis felis. Despite the perceptions of some pet owners, fleas found on cats are rarely rabbit, hedgehog or other fleas.

Eggs, laid by the adult cat flea on the animal, fall off into the environment and so the immature stages are found developing in the environment. Eventually the mature fleas emerges from the cocoon and seeks a host. The time taken for development to the adult is highly dependent on environmental temperature and humidity and may take as little as two weeks or more than four months. An adult female flea is very fecund, so finding one or more fleas on an animal usually means that there are many more developing in the animal's environment.

CURRENT METHODS FOR FLEA CONTROL

Flea control includes grooming of animals to detect and remove fleas, hoovering to remove the immature stages from the environment and chemical reduction of flea numbers. Grooming and hoovering alone are rarely sufficient to control flea

infestations, so flea control products are generally required. These can be considered in two groups: those aimed at killing the adult flea on the animal and those controlling the immature stages in the environment.

The chemical groups used in adulticidal products currently include the pyrethroids, organophosphates, carbamates and, the most recent addition, a phenyl pyrazole compound (fipronil, Frontline, Rhone Merieux). Recent concern over the use of organophosphate sheep dips has led to some reluctance amongst pet owners and veterinarians to use organophosphates on pets. Cats tend to be more susceptible than dogs to organophosphates and pyrethroids, this has tended to limit the number of products available that are licenced for use in the cat. Fleas developing resistance to chemical groups has not been a reported feature in the UK, however it has been widely recognised overseas, most notably in the south US. It is, therefore, important that as many steps as possible are taken to prevent resistance confounding our attempts to control fleas. One such step is to have a variety of unrelated chemical groups available, thereby avoiding reliance on, and hence overuse of, a single chemical group.

Currently there are a large number of formulations and presentations for adulticidal products. For example, products can be applied to the animal in the form of sprays, shampoos, collars, powders, foams or spot-ons. There is also one product for oral administration. Formulation and presentation are important for a number of reasons. Firstly, good owner compliance is essential for effective flea control and owners may be reluctant to complete or repeat treatment if administration is messy, time consuming or if the animal resents treatment. Cats are noted for resenting the sound of an aerosol, making aerosol usage difficult for flea treatment. The spot-on treatments are the quickest and easiest to apply to a cat. Formulation, together with the active ingredient, determine the residual control that the product possesses. Residual control ensures that flea repopulation and reproduction is not possible between treatments. Whilst all products, except for shampoos, will show some residual control of fleas, some of the current products have a tailing-off of efficacy prior to the recommended retreatment date, this may provide a "window" where fleas can reproduce and thus maintain their existence despite treatment. Therefore, current recommendations often suggest using environmental control together with adulticidal treatment. This means that flea control becomes expensive, particularly in a multi-animal household, and time-

consuming. This is another factor that may reduce owner compliance and therefore the effectiveness of a control strategy.

Products aimed at controlling the environmental stages of the flea are either insecticides applied into the environment, insect growth regulators applied into the environment or chitin synthesis inhibitors. The only chitin synthesis inhibitor currently available (lufenuron, Program, Ciba Geigy) is administered orally to the cat and prevents fertile eggs being laid. There will be a lag period after treament with lufenuron is commenced and before control of a flea population is attained as lufenuron as no effect on larvae, pupae or adults. Moreover, it will have no effect on adult fleas that the animal may pick up from the external environment. Flea larvae migrate down to the base of carpets and thereby protect themselves physically from other agents that are applied to the surface of the carpet. Insect growth regulators only affect the early stages of development, leaving the fully developed pupa unharmed. These factors are recognised as providing more "windows of opportunity" for fleas to survive in the face of flea control measures. Overall, therefore, whilst environmental products are a very useful adjunct to flea control, they are incapable of producing immediate and effective flea control. As mentioned before, this means that concurrent adulticide and environmental flea control is frequently recommended. As with the adulticidal products, resistance to the chemicals for envirronmental control products has been reported in other parts of the world so these products, too, must be used rationally to preserve their usefulness.

Altogether this means that, despite all the products available, the flea problem persists and perhaps increases. No one product or group of products has been demonstrated to totally control the problem. There is, therefore, a need for new and better products, particularly for the treatment of cats and especially if they belong to a different chemical group to those currently available.

THE PRODUCT PROFILE - IMIDACLOPRID

The active ingredient, Imidacloprid, is a light yellow solid that has been dissolved in benzyl alcohol and propylene carbonate to produce a pale yellowish-brownish, slightly viscous liquid. The formulation is bitter to taste. The product is applied as a "spot-on". The final formulation contains, 10% w/v (9,1% w/w) imidocloprid

and is presented in 0.4 ml and 0.8 ml pipettes designed for a single application to the skin of the cat. The recommended dose rates are:

< 4 kg	bodyweight	1 x 0.4 ml pipette: "40"
> 4 kg	"	1 x 0.8 ml pipette: "80"

The contents of the pipette are applied to the skin of the cat in the dorsal midline at the base of the skull.

The product is recommended for monthly application to control or prevent flea (Ctenocephalides felis) infection in cats. The product is recommended for treatment of weaned kittens and adult cats. The technical problems of treating unweaned kittens are surmounted by recognition that treatment of the queen results in eliminaiton of infection from both the queen and the kittens.

Each tube or pipette is opened using the same cunning device as that on fenthion (Tiguvon, Bayer) pipettes i.e. a small alan key device in the lid of the pipette opens the sealed end of the pipette. This is both a safety feature and a convenient way of ensuring that the cat owner can open each pipette without spilling any of the contents either onto themselves or into the environment.

PRE-CLINICAL DOCUMENTATION

PHARMACOLOGY AND MODE OF ACTION

Imidacloprid is applied as a "spot on" which then spreads rapidly across the skin of the animal from the point of application. The speed of spreading was demonstrated by concentrations of 27.1 µg Imidacloprid per g hair on the lateral aspect of the thigh of the cats 1 day post treatment with 10% Imidacloprid administered as a single spot on the skin at the base of the skull. Thereafter, its adherence to the hair and probably skin ensures its sustained activity. Twenty-eight days post treatment mean concentrations of 1.1 µg and 1.6 µg Imidacloprid per g hair was measured on the fur taken from the shoulder and side of the thigh, respectively. Imidacloprid then acts on contact by blocking post-synaptic nicotinic receptors in insects.

TOLERANCE OF IMIDACLOPRID SPOT ON 10% BY CATS

A number of studies have been carried out by Bayer investigators, in various parts of the world, to investigate the tolerance of Imidacloprid by cats. A 10% formulation of Imidacloprid, identical to the formulation submitted for approval was used in all of these studies.

The product is applied as a single spot onto the skin. No problems have been observed at the site of application, either in the studies where this was a specific parameter for measurement or in any of the other studies. KERWICK and YOUNG have noted an "oily patch" on the surface of the skin after application up to 24 hours post-treatment. In further study, a very mild, transient (gone by 3 days post-application) discoloration was noted on the coats of white dogs.

A total of ten 6- to 10-week-old kittens were treated with between 142.9 and 240 mg/kg. Four of the kittens were treated twice, with a two week interval between the treatments. One kitten treated with 240 mg/kg Imidacloprid on the back of the neck. Its shoulder blades managed to lick the formulation as it ran down the neck of the animal, due to the large volume. This kitten was observed to salivate for the 15 minutes.. The following day this kitten was sneezing and had a clear, unilateral nasal discharge. All signs cleared uneventfully. No other adverse effects were noted and the kittens continued to grow. Infact in the study, the mean post-treatment growth of one group of treated kittens exceeded that of the controls. Three cats that were treated once daily for three days with 50 mg/kg body weight Imidacloprid has elevated creatine kinase (CPK) levels when sampled 5 days after the final treatment. There was no post-treatment elevation of CPK in two subsequent studies.

In the first study blood samples were collected from six cats two days after the last of 8 weeks treatments, each at 50 mg/kg body weight Imidacloprid. The second study was conducted. Ten cats were treated with 100 mg/kg Imidacloprid. The cats were observed closely and blood samples collected 6, 24, 48 and 72 hours and 7 days post-treatment for CPK estimation. There was no change in the mean creatine kinase level at any of the time-points, compared to the pre-treatment levels.

No interactions with other commonly used veterinary medicines were observed in a study where cats were treated concurrently with Imidacloprid and a veterinary anthelmintic (praziquantel and pyrantel, Drontal, Bayer) or an ectoparasite control measure (lufenuron, Program, Ciba Geigy). Additionally, a wide variety of other veterinary medicines were used concurrently with Imidacloprid treatments in the two field studies. No interactions were noted by the investigators in any of these studies.

Three queens were treated twice during pregnancy with between 39.9 and 41.2 mg/kg on each occasion. The earliest treatment took place on day 6 of pregnancy. Their kittens were observed from birth until between seven and nine days after birth. No abnormalities were observed and the kittens grew as would be expected. Three further queens were treated three times, at between 39.8 and 41 mg/kg, during lactation. Again, no adverse effects were observed either in the queen or kittens.

In addition to the cats treated in the course of the specific tolerance studies, a total of 477 other cats have been treated at least once with Imidacloprid, 467 of these within the therapeutic dose-range, in the course of the efficacy studies. No adverse reactions have been noted in these cats. The reader is directed to the toxicological expert report for further consideration of the toxicology of Imidacloprid to the target species, the environment or humans.

EFFICACY AGAINST A RESISTANT FLEA POPULATION

A preliminary formulation of Imidacloprid was tested against a multiresistant flea strain ("cottontail") imported into Germany from Florida. The fleas were found to be susceptible to Imidacloprid but insusceptible to HCH, carbamates, OPs, rotenone, pyrethrin and synthetic pyrethroids.

CLINICAL DOCUMENTATION

THE EFFICACY OF IMIDACLOPRID AGAINST FLEAS ON CATS

A number of studies have been conducted by investigators on behalf of Bayer, firstly to arrive at a 10% spot-on formulation and 10 mg/kg as a minimum dose-rate. The performance of this dose-rate was then assessed in a series of dose-titration/confirmation studies. In all of these studies, dogs or domestic cats were artificially infected with C. felis. Finally, two field studies were conducted, using Imidacloprid to control natural C. felis infections.

The final formulation was used in all studies except in the initial dose-titration study.

The presence of a clear positive dose-effect relationship, with increased and prolonged duration of efficacy with increased dose-rates was demonstrated in the dose-titration study conducted in dogs.

A dose-rate of 7.5 mg/kg gave good knockdown protection, but efficacy diminised before 28 days. A 10 mg/kg dose-rate produced consistently good knockdown efficacy and duration efficacy up to 28 days. Beyond 28 days after treatment with 10 mg/kg, an increased proportion of dogs retained live fleas following challenge and the numbers surviving on individual dogs increased. Therefore 10 mg/kg was adopted as a rational minimum dose-rate. A 10% solution meant that even in the biggest dogs only 2.5 ml was applied at one location. This was observed to disappear rapidly into the coat and never "run-off" the animal. This was therefore adopted as the concentration in the final formulation.

These findings in dogs were used as the basis for studies to assess the most appropriate dose-range in cats. An initial dose-titration/confirmation study was carried out. 10% Imidacloprid at a dose-rate of 7.5 mg/kg applied between the shoulder blades was seen, as in dogs, to produce good knockdown efficacy at 24 hours post-treatment, but by day 28 protection had decreased to a 56.5% reduction in flea numbers on cats 24 hours post-challenge. One study was conducted to examine the efficacy of 10 mg/kg 10% Imidacloprid applied between the shoulder blades. Good control was seen 24 hours post-treatment, but the efficacy 24 hours after challenge at 28 days post-treatment was somewhat diminished (72.6%).

It was observed that the duration of a high level of protection from flea challenge was shorter than that seen in dogs. It was suggested that, as cats are more fastidious groomers than dogs, possibly they were just removing enough of the application post-treatment to compromise long term protection. The investigators, therefore, moved the location of treatment to a more inaccessible location, namely to the back of the neck at the base of the skull. A series of dose and location of administration confirmation studies were then carried out. These studies showed a consistently higher level of efficacy.

Flea reduction 24 hours after challenge 28 days post-treatment with 10% 10 mg/kg Imidacloprid on the base of the neck was 95.7% and 86.1%.

Good efficacy (89.1%) was obtained after challenge 28 days post-treatment in one study, where cats were treated on the back of the neck according to the proposed therapeutic dose schedule.

The numbers of fleas dead by 24 hours post-treatment or post-challenge was compared with the reduction in numbers 48 hours post-treatment or post-challenge. The results that were obtained:

	weeks post-treatment				
	0	1	2	3	4
1 day	99.8	100	99.3	96.9	86.1
2 days	100	100	100	99.7	96.7

indicate that almost all fleas were killed by 48 hours post-treatment or challenge up to and including fours weeks post-treatment. The effect of treatment of cats with 10% 10 mg/kg imidacloprid on flea egg production was examined.

Percentage reduction in flea eggs in the treated cats compared to the control cats was as follows:

	weeks post-treatment				
	0	1	2	3	4
2 days	98.5	100	99.9	99.9	97.8

indicating that almost all fleas were prevented from laying eggs for four weeks post-treatment.

The effectiveness of 10% 10 mg/kg imidacloprid treatment of queens in order to control flea infestations in their unweaned kittens was investigated. The queens were treated on the back of the neck when the kittens were between 3 and 5 days of age. Treatment was 100% effective by day 1 and kittens were protected from flea challenge thereafter for between 15 and 22 days.

Two field studies were conducted where cats were treated on the back of the neck according to the proposed therapeutic dose-bands. In one study, the performance of imidacloprid was compared to that of fipronil (Frontline). A total of 64 cats were treated with imidacloprid at the proposed therapeutic dose-rates and 59 with fipronil according to the manufacturers' instructions. Eight animals were lost to follow up, so a total of 60 and 55 data-sets were analysed from imidacloprid and fibronil groups, respectively. The majority of cats in both groups were domestic shorthair, with a small number of Siamese and Persian cats in both groups. 80% of the imidacloprid treated cats had hair < 4.5 cm long the remainder had longer hair. There was a similar range of hair-lengths in the fipronil group. The following overall efficacy values (calculated by comparing flea counts to the day 0 values)

day post-treat	IMIDACLOPRID	FIPRONIL
1	87.7	92.1
14	95.4	96.6
21	91.4	95.0
28	88.6	92.7
35	85.5	87.6

A total of 385 cats were treated in the German field study. Seven further cats remained as untreated controls. Cats of both sexes and a variety of breeds and hair types were treated in this study. Of 385 cats 57 were re-assessed after one week for reduction in flea numbers. 47 (82%) were free of fleas and the remainder had moderate to medium infestations. 168 cats were re-assessed 4 weeks post-treatment, of these 140 (83%) were free of fleas and 25 of the remainder showed a markedly reduced infestation.

- Report (1) demonstrates that the compounds in question are systemically active when taken up by animals orally. But their duration of action (about 7 days) is by far less than satisfactory. It requires constant treatment every 7 days for at least 4 weeks, a compliance problem for the owner and the treated animal.

- Report (2) demonstrates that the active compound Imidacloprid when used in form of a spot-on formulation is distributed within 5 days all over the animal. Distribution is not the result of a systemic mode of action via passing through the skin, passing into blood circulation system and oral uptake by the blood sucking flea. This is demonstrated by the fact that Imidacloprid has nearly no effect upon fleas on skin-parts which have been shorn and shaven and cleaned after treatment. Imidacloprid however has full activity on skin parts which have been shorn and shaven but not cleaned after treatment or which remained as they were after treatment.

- Report (3) is the opinion of the expert appointed by Bayer summarizing Bayer's work with Imidacloprid against fleas on cats for registration purposes. This report is included to demonstrate that the concept upon which the present invention is based on is really practically working.

The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Monheim, Germany,
this 14th day of August 1996

Hubert Dorn

(Dr.Hubert Dorn)

Report 1

A comparative clinical examination of the activity and tolerance of oral formulations of imidacloprid and three additional chemically analogous substances by experimentally infesting dogs with fleas

The aim of the present investigation is to examine the activity and tolerance of oral formulations of four chloronicotinyl guanidines using dogs as the target animal species, a fixed dosage and five test groups.

The tests were carried out on 12 dogs (beagles). Since four substances were tested, each test group consisted of 3 animals. 4 additional animals, which were used as controls, had been pretreated with pyriproxyfen.

The following table summarises the animal data relevant for the performance of the study.

Table 1: Animal data

animal no.	sex	weight (kg)
NYAA 255 4283	m	13.4
NYAA 253 2522	m	12.3
NYAA 254 5136	m	13.6
NYAA 254 7104	f	13.7
NYAA 254 9018	f	11.6
NYAA 254 7899	f	10.5
NYAA 255 5042	m	11.2
NYAA 253 1640	m	13.4
NYAA 255 3376	m	12.1
NYAA 254 4768	m	14.1
NYAA 255 4674	f	10.9
NYAA 255 1039	f	10.5
93 4875	f	13.1
94 5732	m	12.6
93 4876	f	13.2
93 4794	f	15.2

The animals were randomised on the basis of the degree of their infestation with fleas. For this purpose the animals were infested with fleas twice before the treatment. On test day 0 the dogs were classified according to the degree of their infestation with fleas and distributed evenly among the various test groups. The control animals were selected according to the degree of their infestation with fleas.

At specific intervals before and after the treatment each dog was infested with approximately 100 fleas [cat fleas, C. felis (Siphonaptera: Pulicidae) - unfed imagoes no more than 4 weeks old] in the inside thigh area. The acceptance by the host was monitored. The corresponding intervals at which the individual preinfestations and the subsequent reinfestations were carried out are shown in the table at the end of this chapter.

The test substances were administered once in the form of tablets each containing 20 mg of active comopund. Test group1 was treated with an oral formulation of imidacloprid, and a corresponding formulation of each of the substances TI 304, TI 435 and AKD 1022 was administered to test groups 2 to 4. A fifth test group was used as a control group.

In order to obtain a more precise comparison of the test substances the dosage was uniformly set at 10 mg/kg body weight.

The following table lists the groups into which the animals were divided and the dosages of the corresponding substances administered to each individual animal.

Table 2: Overview of the test groups and the treatment employed

test group	animal no.	weight (kg)	dosage	
			mg	no. of tablets
imidacloprid	NYAA 255 4283	13.4	130	6.5
	NYAA 253 2522	12.3	120	6
	NYAA 254 5136	13.6	140	7
TI 304	NYAA 254 7104	13.7	140	7
	NYAA 254 9018	11.6	120	6
	NYAA 254 7899	10.5	110	5.5
TI 435	NYAA 255 5042	11.2	110	5.5
	NYAA 253 1640	13.4	120	6
	NYAA 255 3376	12.1	120	6
AKD 1022	NYAA 254 4768	14.1	140	7
	NYAA 255 4674	10.9	110	5.5
	NYAA 255 1039	10.5	110	5.5
control	93 4875	13.1	-	-
	94 5732	12.6	-	-
	93 4876	13.2	-	-
	93 4794	15.2	-	-

The test substances to be examined belong to the nicotinyl nitroguanidine class of compounds. The substances were in the form of 120 mg tablets each containing 20 mg of active compound.

I. imidacloprid 1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylidene amine

II. TI 304 N-[(6-chloro-3-pyridinyl)methyl]-N-ethyl-N-methyl-2-nitro-1,2-ethene diamine

III. TI 435 1-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

IV. AKD 1022 1-[(2-chloro-5-thiazolyl)methyl]-tetrahydro-3,5-dimethyl-N-nitro-1,3,5-triazin-2-imine

In order to examine the activity and tolerance of the treatment all the animals of the individual treatment groups and the control groups were subjected to a general clinical examination and a special clinical examination for their infestation with fleas 24, 48 and 72 hours after the treatment. This procedure was repeated after the weekly reinfestation (see Table 3). Tolerance was assessed qualitatively (individual description of the findings determined).

Table 3: The time schedule of the tests

1: test group 1 (imidacloprid) 2: test group 2 (TI 304) 3: test group 3 (TI 435)
4: test group 4 (AKD 1022) 5: test group 5 (control)

day of test	date	determination of weight	randomisation	infestation	treatment	clinical examination
-7	12.3.96			1, 2, 3, 4, 5		
-3	15.3.96			1, 2, 3, 4, 5		
-1	18.3.96	1, 2, 3, 4, 5				
0	19.3.96	1, 2, 3, 4, 5	1, 2, 3, 4, 5		1, 2, 3, 4, 5	1, 2, 3, 4, 5
1	20.3.96					1, 2, 3, 4, 5
2	21.3.96					1, 2, 3, 4, 5
3	22.3.96					1, 2, 3, 4, 5
7	26.3.96			1, 2, 3, 4, 5		
8	27.3.96					1, 2, 3, 4, 5
9	28.3.96					1, 2, 3, 4, 5
10	29.3.96					1, 2, 3, 4, 5

Results and discussion

All of the substances tested displayed a very high immediate activity. On test day 1 (24 hours after the administration of the substances) all the animals in the treatment groups were free from fleas.

The flea infestation rates on test day 8 (24 hours after reinfestation) indicate that only a small degree of residual activity exists which no longer produces any major reduction in the infestation of the treated animals with fleas. Also the activity between day 8 (24 hours after reinfestation) and day 10 (72 hours after reinfestation) no longer reveals any insecticidal activity of the substances tested.

The clinical tolerance of the oral formulations was very high. The clinical examination of the animals did not provide any indication of intolerance reactions.

The duration of activity is therefore between 1-7 days for all of the substances. The assessment of the duration of activity could only be carried out approximately for methodological reasons, since reinfestation was not carried out until 7 days after the treatment. No major differences in activity or tolerance were determined between the individual substances.

All the results obtained relate to the formulations tested with a dosage of 10 mg/kg. It is thus unlikely that adequate protection against infestation with fleas can be guaranteed by a single dose of <10mg/kg of the formulations in question.

Report 2

The spot-on mode of action of 10% spot-on imidacloprid against the infestation of dogs with fleas (Ctenocephalides felis)

On day 0, 3 beagles were each treated dermally between their shoulder blades with 0.1 ml/kg body weight of 10% spot-on imidacloprid (10 mg/kg body weight). 3 additional beagles were not treated and were used as control dogs.

On day 5, 3 flea cages containing cat fleas were attached to specially prepared areas of skin of the thorax region of each of the 6 beagles:

I one flea cage per dog was attached directly to skin which had been cleaned with propylene carbonate, washed with soap and then shorn and shaven;

II one flea cage per dog was attached directly to shorn and shaven skin which had not be cleaned with propylene carbonate or washed;

III one flea cage per dog was attached to fur or skin which had not been cleaned with propylene carbonate or washed.

On day 6, 7 and 8 the fleas were examined for their vitality and sucking activity.

The following observations were made with regard to the fleas on the dogs treated with imidacloprid, 3 days after they had been applied: Those fleas on skin (I) which was substantially free of imidacloprid were only slightly affected after 3 days on the host (their movements were slower than on the control dogs) and a large amount of blood had been sucked (although not as much as in the control dogs). After 3 days almost no blood had however been sucked by the fleas on the skin (II) containing imidacloprid or on the fur

and skin(III) containing imidacloprid and the mortality was almost 100% (the few fleas which were still alive were severely affected).

In all three cases (I; II and III) the fleas on the control dogs had not been affected at all and had sucked a large amount of blood after 3 days.

beagles	flea cage	day 6 (24 h after the attachment of the flea cages)	day 7 (48 h after the attachment of the flea cages)	day 8 (72 h after the attachment of the flea cages)
3 beagles treated with imidacloprid	I	flea excrement + all of the fleas alive	flea excrement ++ all of the fleas alive	flea excrement +++ all of the fleas alive, but slightly affected (they move more slowly than on the control animals)
	II	flea excrement - some of the fleas dead	flea excrement - the majority of the fleas dead	flea excement - (in some cases only very small amounts present) all of the fleas dead (a few fleas are still alive, but they are severely affected)
	III	flea excrement - no fleas visible in the fur	flea excrement - no fleas visible in the fur	flea excrement - (in some cases only very small amounts present) all of the fleas dead (a few fleas are still alive, but they are severely affected)
3 untreated control dogs	I	flea excrement ++ all of the fleas alive	flea excrement +++ all of the fleas alive	flea excrement ++++ all of the fleas alive and unaffected
	II	flea excrement ++ all of the fleas alive	flea excrement +++ all of the fleas alive	flea excrement ++++ all of the fleas alive and unaffected
	III	flea excrement ++ no fleas visible in the fur	flea excrement +++ no fleas visible in the fur	flea excrement ++++ all of the fleas alive and unaffected

I = fleas on shorn, shaven skin which has been cleaned with propylene carbonate and washed with soap

II = fleas on shorn, shaven skin which has not been cleaned with propylene carbonate or washed

III = fleas on hairy skin which has not been cleaned with propylene carbonate or washed

On day 0, 3 beagles were each treated dermally between the shoulder blades with 0.1 ml/kg of 10% spot-on imidacloprid (10 mg/kg). 3 additional beagles were not treated and were used as controls. On day 5, 3 flea cages

containing cat fleas were attached to specially prepared areas of skin of the thorax region of each of the 6 beagles:

I one flea cage per dog was attached directly to skin which had been cleaned with propylene carbonate and washed with soap and then shorn and shaven;

II one flea cage per dog was attached directly to shorn and shaven skin which had not be cleaned with propylene carbonate or washed;

III one flea cage per dog was attached to fur or skin which had not been cleaned with propylene carbonate or washed.

On day 6, 7 and 8 the fleas were examined for their vitality and sucking activity.

The fleas in flea cages I, II and III on the control dogs were not affected at all and displayed a high degree of sucking activity over the period of observation.

The fleas in flea cage 1 on the dogs treated with imidacloprid were only slightly affected and displayed a high degree of sucking activity over the period of observation.

By contrast, the fleas in flea cages II and III on the dogs treated with imidacloprid displayed a mortality of almost 100 % at the end of the period of observation and practically no excrement had been deposited.

These results reveal that the active compound present on the skin and in the fur of animals plays a crucial role. The mere presence of imidacloprid on the skin seems to be sufficient to ensure a 100 % mortality of the fleas (there is no significant difference between the fleas in flea cages II and III). The

amount of imidacloprid merely consumed orally by the fleas via the blood does not appear to be sufficient to ensure the destruction of the fleas within 3 days. Thus 10% spot-on imidacloprid appears to have very high contact activity on being applied dermally to dogs. This is above all due to the active compound present on the skin, whereas the oral effect is minimal.

The slight effect on the fleas which was nevertheless observed in flea cages I on the dogs treated with imidacloprid - including the smaller amount of blood sucked compared with the controls - could be due to imidacloprid still being present on the skin, despite cleaning. It is however also possible that the quantity of active compound consumed orally by the fleas after sucking blood for three days is sufficient to affect the fleas slightly.

Report 3

EXPERT REPORT ON THE PRE-CLINICAL AND CLINICAL DOCUMENTATION

ASSESSMENT OF THE USE OF IMIDACLOPRID TO PREVENT AND TREAT FLEA INFESTATION, CAUSED BY *Ctenocephalides felis felis* AND *Ctenocephalides canis*, IN CATS

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Signed.....

Dated...27th April 1996.....

ASSESSMENT OF THE USE OF IMIDACLOPRID TO PREVENT AND TREAT FLEA INFESTATION CAUSED BY *Ctenocephalides felis felis* IN CATS

INTRODUCTION

Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine) is a chloronicotinyl nitroguanidine compound that was originally developed as an insecticide for plant use. It was not originally recognised as possessing useful pulicidal properties when it was screened in-vitro. However, in 1994 **HOPKINS & HEDEMANN (1994)** obtained excellent flea kill when an imidacloprid suspension was sprayed onto flea-infested cats. That observation led to the development of the formulation presented here. p.32 ref.no.1

This clinical expert report has been written to assess the tolerance and efficacy profile of imidacloprid, in the proposed final formulation, for the treatment and control of fleas on cats.

THE FLEA PROBLEM

Fleas on domestic pets are becoming recognised as an increasing problem, despite the availability of numerous "flea remedies". There are several possible reasons for this, firstly that there is a growing cat and dog population. For example, the Pet Food Manufacturers Association in the UK reported that the cat population had increased by 33% between 1981 and 1991. During the same time the dog population had increased by 22%. Many of these dogs and cats live in our homes which, especially with central heating, are now maintained at higher ambient temperatures than was previously possible. Additional features such as fitted carpets, upholstered furniture and draught-proofing

have been cited as factors that may assist in maintaining flea populations in domestic accomodation (KRISTENSEN, HAARLOV AND MOURIER, 1978). Finally, recent mild, humid winters have prevented flea numbers from being drastically reduced, as occurs in dry, cold weather. Therefore, after such mild winters, there is a substantial baseline population (OLSEN, 1990). p.65 p.84

The majority of skin conditions seen in small animal, first opinion practice are related to flea infestation. The most commonly observed clinicial sign of flea infestation in cats (and dogs) is caused by the development of an allergy to flea saliva, injected as the fleas bit, resulting in flea allergic dermatitis. This is a distressing skin condition which, as far as can be ascertained from the current literature, will flare up again in a sensitised individual whenever that individual is exposed to a threshold number of fleas. It is therefore crucial to adopt a flea population reduction policy in these situations. Fleas are also responsible for the transmission of diseases, for example the flea acts as an intermediate host for the tapeworm, *Dipylidium caninum*.

Fleas, particularly unfed cat (*Ctenocephalides felis felis*), are zoonoses as they will bite humans. Some humans develop a hypersensitivity reaction to these bites in a similar way to dogs or cats (MAUNDER, 1995). Fleas may also transmit disease to humans, for example the literature contains reports of human infection with *Dipylidium caninum*. It may therefore be appreciated that, apart from any aesthetic concerns for avoiding having flea-infested pets, fleas can pose a threat to animal and human health. p.64

The most commonly found flea on cats is the cat flea, *Ctenocephalides felis felis*. Despite the perceptions of some pet owners, fleas found on cats are rarely rabbit, hedgehog or other fleas.

Eggs, laid by the adult cat flea on the animal, fall off into the environment and so the immature stages are found developing in the environment. Eventually the mature flea emerges from the cocoon and seeks a host. The time taken for development to the adult is highly dependent on environmental temperature and humidity and may take as little as two weeks or more than four months. An adult female flea is very fecund, so finding one or more fleas on an animal usually means that there are many more developing in the animal's environment.

CURRENT METHODS FOR FLEA CONTROL

Flea control includes grooming of animals to detect and remove fleas, hoovering to remove the immature stages from the environment and chemical reduction of flea numbers. Grooming and hoovering alone are rarely sufficient to control flea infestations, so flea control products are generally required. These can be considered in two groups: those aimed at killing the adult flea on the animal and those controlling the immature stages in the environment.

The chemical groups used in adulticidal products currently include the pyrethroids, organophosphates, carbamates and, the most recent addition, a phenyl pyrazole compound (fipronil, Frontline, Rhone Merieux). Recent concern over the use of organophosphate sheep dips has led to some reluctance amongst pet owners and veterinarians to use organophosphates on pets. Cats tend to be more susceptible than dogs to organophosphates and pyrethroids, this has tended to limit the number of products available that are licenced for use in the cat. Fleas developing resistance to chemical groups has not been a reported feature in the UK, however it has been widely recognised overseas, most notably in the south US. It is, therefore, important that as many

steps as possible are taken to prevent resistance confounding our attempts to control fleas. One such step is to have a variety of unrelated chemical groups available, thereby avoiding reliance on, and hence overuse of, a single chemical group.

Currently there are a large number of formulations and presentations for adulticidal products. For example, products can be applied to the animal in the form of sprays, shampoos, collars, powders, foams or spot-ons. There is also one product for oral administration. Formulation and presentation are important for a number of reasons. Firstly, good owner compliance is essential for effective flea control and owners may be reluctant to complete or repeat treatment if administration is messy, time consuming or if the animal resents treatment. Cats are noted for resenting the sound of an aerosol, making aerosol usage difficult for flea treatment. The spot-on treatments are the quickest and easiest to apply to a cat. Formulation, together with the active ingredient, determine the residual control that the product possesses. Residual control ensures that flea repopulation and reproduction is not possible between treatments. Whilst all products, except for shampoos, will show some residual control of fleas, some of the current products have a tailing-off of efficacy prior to the recommended retreatment date, this may provide a "window" where fleas can reproduce and thus maintain their existence despite treatment. Therefore, current recommendations often suggest using environmental control together with adulticidal treatment. This means that flea control becomes expensive, particularly in a multi-animal household, and time-consuming. This is another factor that may reduce owner compliance and therefore the effectiveness of a control strategy.

Products aimed at controlling the environmental stages of the flea are either insecticides applied into the environment, insect growth regulators applied into the

environment or chitin synthesis inhibitors. The only chitin synthesis inhibitor currently available (lufenuron, Program, Ciba Geigy) is administered orally to the cat and prevents fertile eggs being laid. There will be a lag period after treatment with lufenuron is commenced and before control of a flea population is attained as lufenuron has no effect on larvae, pupae or adults. Moreover, it will have no effect on adult fleas that the animal may pick up from the external environment. Flea larvae migrate down to the base of carpets and thereby protect themselves physically from other agents that are applied to the surface of the carpet. Insect growth regulators only affect the early stages of development, leaving the fully developed pupa unharmed. These factors are recognised as providing more "windows of opportunity" for fleas to survive in the face of flea control measures. Overall, therefore, whilst environmental products are a very useful adjunct to flea control, they are incapable of producing immediate and effective flea control. As mentioned before, this means that concurrent adulticide and environmental flea control is frequently recommended. As with the adulticidal products, resistance to the chemicals for environmental control products has been reported in other parts of the world so these products, too, must be used rationally to preserve their usefulness.

Altogether this means that, despite all the products available, the flea problem persists and perhaps increases. No one product or group of products has been demonstrated to totally control the problem. There is, therefore, a need for new and better products, particularly for the treatment of cats and especially if they belong to a different chemical group to those currently available.

THE PRODUCT PROFILE - IMIDACLOPRID

The active ingredient, Imidacloprid, is a light yellow solid

that has been dissolved in benzyl alcohol and propylene carbonate to produce a pale yellowish-brownish, slightly viscous liquid. The formulation is bitter to taste. The product is applied as a "spot-on". The final formulation contains 10%w/v (9.1%w/w) imidocloprid and is presented in 0.4ml and 0.8ml pipettes designed for a single application to the skin of the cat. The recommended dose rates are:

<4kg	bodyweight	1 x 0.4 ml pipette: "40"
>4kg	"	1 x 0.8 ml pipette: "80"

The contents of the pipette are applied to the skin of the cat in the dorsal midline at the base of the skull.

The product is recommended for monthly application to control or prevent flea (*Ctenocephalides felis*) infection in cats. The product is recommended for treatment of weaned kittens and adult cats. The technical problems of treating unweaned kittens are surmounted by recognition that treatment of the queen results in elimination of infection from both the queen and the kittens (KERWICK, 1996). p.61, ref.no.20

Each tube or pipette is opened using the same cunning device as that on fenthion (Tiguvon, Bayer) pipettes ie a small alan key device in the lid of the pipette opens the sealed end of the pipette. This is both a safety feature and a convenient way of ensuring that the cat owner can open each pipette without spilling any of the contents either onto themselves or into the environment.

PRE-CLINICAL DOCUMENTATION

PHARMACOLOGY AND MODE OF ACTION

Imidacloprid is applied as a "spot on" which then spreads rapidly across the skin of the animal from the point of

application. The speed of spreading was demonstrated by **FICHTEL & ALTGASSEN (1996)** who found concentrations of 27.1µg Imidacloprid per g hair on the lateral aspect of the thigh of the cats 1 day post treatment with 10% Imidacloprid administered as a single spot on the skin at the base of the skull (see figure). Thereafter, its adherence to the hair and probably skin ensures its sustained activity. Twenty-eight days post treatment mean concentrations of 1.1µg and 1.6µg Imidacloprid per g hair was measured on the fur taken from the shoulder and side of the thigh, respectively. Imidacloprid then acts on contact by blocking post-synaptic nicotinic receptors in insects. p.33, ref.no. 2

TOLERANCE OF IMIDACLOPRID SPOT ON 10% BY CATS

A number of studies have been carried out by Bayer investigators, in various parts of the world, to investigate the tolerance of imidacloprid by cats. A 10% formulation of imidacloprid, identical to the formulation submitted for approval was used in all of these studies.

The product is applied as a single spot onto the skin. No problems have been observed at the site of application, either in the studies where this was a specific parameter for measurement (see Tables 1-3) or in any of the other studies. **KERWICK (1995)** and **YOUNG (1995)**, have noted an "oily patch" on the surface of the skin after application up to 24 hours post-treatment. In further study **KERWICK (1995)** noted a very mild, transient (gone by 3 days post-application) discoloration was noted on the coats of white dogs. p.23-25 p.41, ref.no. 9 p.59, ref.no.19 p42, ref.no.10

The format of each of the weaned kitten and adult cat tolerance studies is summarised in Tables 1 and 2. A total of ten 6- to 10-week-old kittens were treated with between 142.9 and 240mg/kg. Four of the kittens were treated twice,

with a two week interval between the treatments. One kitten treated with 240mg/kg imidacloprid (KERWICK, 1995) on the back of the neck its shoulder blades managed to lick the formulation as it ran down the neck of the animal, due to the large volume. This kitten was observed to salivate for 15 minutes. The following day this kitten was sneezing and had a clear, unilateral nasal discharge. All signs cleared uneventfully. No other adverse effects were noted and the kittens continued to grow. Infact in the study by KERWICK (1995) the mean post-treatment growth of one group of treated kittens exceeded that of the controls. Three cats (SHMIDL & ARTHER, 1995) that were treated once daily for three days with 50 mg/kg body weight imidacloprid had elevated creatine kinase (CPK) levels when sampled 5 days after the final treatment. There was no post-treatment elevation of CPK in two subsequent studies (SHMIDL & ARTHER, 1995; COSTELLO, 1995). In the first study (SHMIDL & ARTHER, 1995) blood samples were collected from six cats two days after the last of 8 weekly treatments, each at 50 mg/kg body weight imidacloprid. The second study was conducted by COSTELLO (1995). Ten cats were treated with 100 mg/kg imidacloprid. The cats were observed closely and blood samples collected 6, 24, 48 and 72 hours and 7 days post-treatment for CPK estimation.There was no change in the mean creatine kinase level at any of the time-points, compared to the pre-treatment levels.

p.41, ref.no.9
p.41, ref.no.9
p.35, ref.no.3
p.36, ref.no.4
p.38, ref.no.6
p.36, ref.no.4
p.38, ref.no.6

No interactions with other commonly used veterinary medicines were observed in a study conducted by SHMIDL & EWALD-HAMM (1995), where cats were treated concurrently with imidacloprid and a veterinary anthelmintic (praziquantel and pyrantel, Drontal, Bayer) or an ectoparasite control measure (lufenuron, Program, Ciba Geigy). Additionally, a wide variety of other veterinary medicines were used concurrently with the imidacloprid treatments in the two field studies (Table 4B). No interactions were noted by the investigators in any of these studies.

p.37, ref.no.5
p.27

Three queens were treated twice during pregnancy with between 39.9 and 41.2mg/kg on each occasion (Table 3). The earliest treatment took place on day 6 of pregnancy. Their kittens were observed from birth until between seven and nine days after birth. No abnormalities were observed and the kittens grew as would be expected (KERWICK, 1996). Three further queens were treated three times, at between 39.8 and 41mg/kg, during lactation. Again, no adverse effects were observed either in the queen or kittens (KERWICK, 1996). p.25 p.39,ref.no. 7 p.40, ref.no.8

In addition to the cats treated in the course of the specific tolerance studies, a total of 477 other cats have been treated at least once with imidacloprid, 467 of these within the therapeutic dose-range, in the course of the efficacy studies. No adverse reactions have been noted in these cats. The reader is directed to the toxicological expert report for further consideration of the toxicology of imidacloprid to the target species, the environment or humans.

EFFICACY AGAINST A RESISTANT FLEA POPULATION.

A preliminary formulation of Imidacloprid was tested against a multiresistant flea strain ("cottontail") imported into Germany from Florida. The fleas were found to be susceptible to Imidacloprid but insusceptible to HCH, carbamates, OPs, rotenone, pyrethrin and synthetic pyrethroids (BARDT & SCHEIN, 1996). p.45, ref.no.13

CLINICAL DOCUMENTATION

THE EFFICACY OF IMIDACLOPRID AGAINST FLEAS ON CATS

A number of studies have been conducted by investigators on behalf of Bayer, firstly to arrive at a 10% spot-on formulation and 10mg/kg as a minimum dose-rate. The performance of this dose-rate was then assessed in a series of dose-titration/confirmation studies. In all of these studies, dogs or domestic cats were artificially infected with *C.felis*. Finally, two field studies were conducted, using imidacloprid to control natural *C.felis* infections. The format of each of these studies is summarised in Tables 4a and 4b. The final formulation was used in all studies except in the initial dose-titration study (**HEESCHEN & LIEBISCH, 1994**) p.53, ref.no.17

The presence of a clear positive dose-effect relationship, with increased and prolonged duration of efficacy with increased dose-rates was demonstrated in the dose-titration study by **HEESCHEN & LIEBISCH, (1994)** conducted in dogs p.53, ref.no.17 (Table 4a). A dose-rate of 7.5mg/kg gave good knockdown protection, but efficacy diminished before 28 days. A 10mg/kg dose rate produced consistently good knockdown efficacy and duration efficacy up to 28 days. Beyond 28 days after treatment with 10mg/kg an increased proportion of dogs retained live fleas following challenge and the numbers surviving on individual dogs increased. Therefore 10mg/kg was adopted as a rational minimum dose-rate. A 10% solution meant that even in the biggest dogs only 2.5ml was applied at one location. This was observed to disappear rapidly into the coat and never "run-off" the animal. This was therefore adopted as the concentration in the final formulation.

These findings in dogs were used as the basis for studies to assess the most appropriate dose-range in cats. An initial dose-titration/confirmation study was carried out by

CUNNINGHAM (1995). 10% imidacloprid at a dose-rate of 7.5mg/kg applied between the shoulder blades was seen, as in dogs, to produce good knockdown efficacy at 24 hours post-treatment, but by day 28 protection had decreased to a 56.5% reduction in flea numbers on cats 24 hours post-challenge. One study was conducted to examine the efficacy of 10mg/kg 10% imidacloprid applied between the shoulder blades **(CRUTHERS, 1995).** Good control was seen 24 hours post-treatment, but the efficacy 24 hours after challenge at 28 days post-treatment was somewhat diminished (72.6 %). p.46, ref.no.14 p.48, ref.no.15

It was observed that the duration of a high level of protection from flea challenge was shorter than that seen in dogs. It was suggested that, as cats are more fastidious groomers than dogs, possibly they were just removing enough of the application post-treatment to compromise long term protection. The investigators, therefore, moved the location of treatment to a more inaccessible location, namely to the back of the neck at the base of the skull. A series of dose and location of administration confirmation studies were then carried out. These studies showed a consistently higher level of efficacy.

Flea reduction 24 hours after challenge 28 days post-treatment with 10% 10mg/kg imidacloprid on the base of the neck was 95.7% and 86.1% in studies by **KERWICK (1995)** and **JACOBS (1995),** respectively. Good efficacy (89.1%) was obtained after challenge 28 days post-treatment in one study **(YOUNG, 1995),** where cats were treated on the back of the neck according to the proposed therapeutic dose schedule. p.50, ref.no.16 p.56, ref.no.18 p.59, ref.no.19

The numbers of fleas dead by 24 hours post-treatment or post-challenge was compared by **JACOBS (1995)** with the reduction in numbers 48 hours post-treatment or post-challenge. The results that were obtained: p.56, ref.no.18

	weeks post-treatment				
	0	1	2	3	4
1 day	99.8	100	99.3	96.9	86.1
2 days	100	100	100	99.7	96.7

indicate that almost all fleas were killed by 48 hours post-treatment or challenge up to and including four weeks post-treatment. The effect of treatment of cats with 10% 10mg/kg imidacloprid on flea egg production was examined by **KERWICK (1995)**. Percentage reduction in flea eggs in the treated cats compared to the control cats was as follows: p.50, ref.no.16

	weeks post-treatment				
	0	1	2	3	4
2 days	98.5	100	99.9	99.9	97.8

indicating that almost all fleas were prevented from laying eggs for four weeks post-treatment.

The effectiveness of 10% 10mg/kg imidacloprid treatment of queens in order to control flea infestations in their unweaned kittens was investigated **(KERWICK, 1996)**. The queens were treated on the back of the neck when the kittens were between 3 and 5 days of age. Treatment was 100% effective by day 1 and kittens were protected from flea challenge thereafter for between 15 and 22 days. p.61, ref.no.20

Two field studies were conducted (Table 4b) where cats were treated on the back of the neck according to the proposed therapeutic dose-bands. In one study conducted by **MERIEUX (1996)** the performance of imidacloprid was compared to that of fipronil (Frontline). A total of 64 cats were treated with imidacloprid at the proposed therapeutic dose-rates and 59 with fipronil according to the manufacturers' instructions. Eight animals were lost to follow up, so a total of 60 and 55 data-sets were analysed from imidacloprid and fibronil groups, respectively. The majority of cats in p.62, ref.no.21

both groups were domestic shorthair, with a small number of Siamese and Persian cats in both groups. 80% of the imidacloprid treated cats had hair <4.5cm long the remainder had longer hair. There was a similar range of hair-lengths in the fipronil group. The following overall efficacy values (calculated by comparing flea counts to the day 0 values) were obtained:

day post-treat	IMIDACLOPRID	FIPRONIL
1	87.7	92.1
14	95.4	96.6
21	91.4	95.0
28	88.6	92.7
35	85.5	87.6

A total of 385 cats were treated in the German field study **(SCHEIN & KRIEGER, 1996)**. Seven further cats remained as untreated controls. Cats of both sexes and a variety of breeds and hair types were treated in this study. Of 385 cats 57 were re-assessed after one week for reduction in flea numbers. 47 (82%) were free of fleas and the remainder had moderate to medium infestations. 168 cats were re-assessed 4 weeks post-treatment, of these 140 (83%) were free of fleas and 25 of the remainder showed a markedly reduced infestation. p.63, ref.no.22